

Strong pOptima[®]-B Cloning Vector

Catalog No. **PVB01**, 1µg (50 ng/µl) : 20 rxns

Product Description

The blunt-end cloning vector pOptima[®]-B is supplied in a linear form, ready-to-use for direct ligation of blunt-end DNA fragment. It is suitable for use in cloning PCR product of proofreading DNA polymerase or other source of blunt-end DNA fragment generated by a blunt-end restriction enzyme. To promote transformation efficiency, the pOptima[®]-B vector was created by blunt-end restriction enzyme digestion and dephosphorylation treatment, thus it could rejoin to a blunt-end insert DNA fragment directly

Procedure

1. Prepare a ligation mixture according to the following scheme :

Component	Concentration	Volume
pOptima [®] -B vector	50 ng/µl	1 µl
Insert DNA		2~7 µl
ligation buffer	10 X	1 µl
T4 DNA ligase	2 U/µl	1 µl
sterile water		5~0 µl
total volume		10 µl

- Briefly mix the ligation mixture then incubate the mixture at room temperature for several hours or at 4°C for overnight (check <http://www.strongbiotech.com> for the relationship between transformation rate and reaction condition).
- Heat the ligation mixture in 50 °C for 10 minutes before transformation.
- Transform competent cells with aliquots of ligation mixture and select transformants on antibiotic-containing media.

Storage:

Available for 1 year when stored at -20 °C.

Notes:

- Generally ligation buffers contain ATP. Frequent freezing and thawing cause ATP hydrolysis. The ligation buffer can be divided into suitable aliquots for storage.
- Mix the thawed ligation buffer well before use. The ligation mixture should be homogenous as well.
- Transformant number formed is proportional to the time of ligation. Overnight transformation at 4°C is suggested.

References:

- Borovkov-AY and Rivkin-MI. (1997) Biotechniques. 22:812-4.
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- Testori-A; Listowsky-I and Sollitti-P. (1994) Gene. 143:151-2.
- Harrison-J; Molloy-PL and Clark-SJ. (1994) Anal-Biochem. 216:235-6.
- Cha-J; Bishai-W and Chandrasegaran-S. (1993) Gene.136: 369-70.

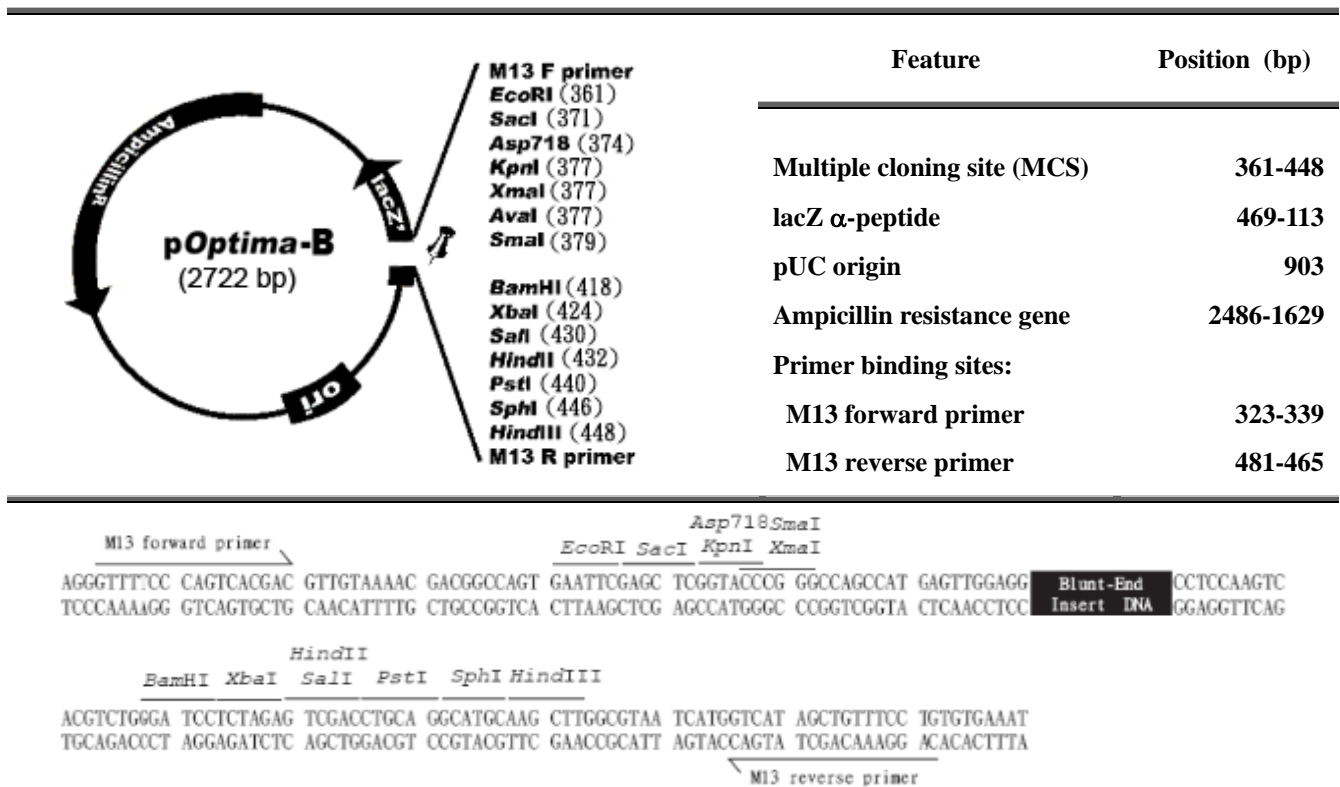


Figure 1. pOptima[®]-B Vector Map. Representation of the linearized pOptima[®]-B vector with blunt-end and dephosphorylation (the pin labeled) for cloning blunt-end insert DNA fragments. The unique restriction endonuclease recognition sites of multiple cloning site are listed. DNA sequence of the region surrounding the multiple cloning site.